

60/087,561, filed June 1, 1998, and is a continuing application of U.S.S.N. 09/127,926, filed July 31, 1998, and 09/058,459, filed April 10, 1998.--

Please replace the paragraph beginning at page 87, line 9, with the following rewritten paragraph:

--**Table 11.** *DEE determined optimal sequences for the core positions of  $G\beta l$  as a function of  $\Delta h_{0.9}^a$* --

Please replace the paragraph beginning at page 88, line 1, with the following rewritten paragraph:

--**Table 12.** *DEE determined optimal sequences for the core positions of  $G\beta l$  as a function of  $\Delta h_{1.0}^a$* --

Please replace the paragraph beginning at page 89, line 1, with the following rewritten paragraph:

--**Table 13.** *DEE determined optimal sequences for the core positions of  $G\beta l$  as a function of  $\Delta Q_{0.9}^a$* --

Please replace the paragraph beginning at page 90, line 1, with the following rewritten paragraph:

--**Table 14.** *DEE determined optimal sequences for the core positions of  $G\beta l$  as a function of  $\Delta \theta_{0.9}^a$* --

Please replace the paragraph beginning at page 91, line 1, with the following rewritten paragraph:

--**Table 15.** *DEE determined optimal sequences for the core positions of  $G\beta l$  as a function of  $\Delta \sigma_{0.9}^a$* --

Please replace the paragraph beginning at page 91, line 21, with the following rewritten paragraph:

--The optimal sequence for the ten core positions of G $\beta$ 1 that is calculated using the native backbone (i.e., no perturbation) contains three conservative mutations relative to the wild-type sequence (Table 11). Y3F and V39I are likely the result of the hydrophobic surface area burial term in the scoring function. L7I reflects a bias in the rotamer library used for these calculations. The crystal structure of G $\beta$ 1 has the leucine at position 7 with a nearly eclipsed  $\chi_2$  of 111°. This strained  $\chi_2$  is unlikely to be an artifact of the structure determination since it is present in two crystal forms and a solution structure (Gronenborn et al., 1991; Gallagher et al., 1994). Our rotamer library does not contain eclipsed rotamers and no staggered leucine rotamers pack well at this position. Instead, the side-chain selection algorithm chose an isoleucine rotamer that conserves the  $\chi_1$  dihedral and is able to pack well. We expect the removal of the strained leucine rotamer to stabilize the protein, a prediction that is tested in the experimental section of this work. The sequences that result from varying individual super-secondary structure parameter values show two notable trends. Small variations in the parameter values tend to have little or no effect on the calculated sequences. For example, varying  $\Delta h_{0.9}$  from -0.25 to -1.00 Å (Table 11) and  $\Delta h_{1.0}$  from +0.25 to +1.25 Å (Table 2) has no effect on the calculated sequences which demonstrates the side-chain selection algorithm's tolerance to small variations in the initial backbone geometry. Large variations in the parameter values tend to result in greater sequence diversity. For example,  $\Delta h_{1.0}[+1.50\text{\AA}]$  contains six out of ten possible mutations relative to G $\beta$ 1 (Table 12). The apparently anomalous result that occurs for  $\Delta h_{0.9}$  at -1.25 and -1.50 Å, an increase in core volume, is explained by the observation that translating the helix towards the sheet plane results in creating a pocket of space in the vicinity of position 20 that ultimately leads to the observed A20V mutation.--

Please replace the paragraph beginning at page 92, line 10, with the following rewritten paragraph:

--Experimental validation of the designed cores focused on seven of the  $\Delta h$ -series mutants which contain between three and six sequence changes relative to G $\beta$ 1. The designed sequences resulting from  $\Delta\Omega$ ,  $\Delta\theta$  and  $\Delta\sigma$  perturbations are, however, in many cases identical to various  $\Delta h$ -series sequences. Typical far UV circular dichroism (CD) spectra are shown in Figure 15.  $\Delta h_{0.9}[-1.00\text{\AA}]$ ,  $\Delta h_{0.9}[0.00\text{\AA}]$ ,  $\Delta h_{0.9}[+0.75\text{\AA}]$  and  $\Delta h_{0.9}[+1.00\text{\AA}]$  have CD spectra that are indistinguishable from that of G $\beta$ 1 while  $\Delta h_{0.9}[+1.50\text{\AA}]$ ,  $\Delta h_{1.0}[+1.50\text{\AA}]$  and  $\Delta h_{0.9}[-1.50\text{\AA}]$  have CD spectra similar to that of G $\beta$ 1 suggesting that all of the mutants have a secondary structure content similar to the wild-type protein. Thermal melts monitored by CD are shown in Figure 16. All of the mutants have cooperative transitions with melting temperatures ( $T_m$ 's) ranging from 53 °C for  $\Delta h_{0.9}[+1.50\text{\AA}]$  to 91 °C for  $\Delta h_{0.9}[0.00\text{\AA}]$  (Table 11). The  $T_m$  for G $\beta$ 1 is 85°C. The measured  $T_m$ 's for  $\Delta h_{0.9}[-1.50\text{\AA}]$  and  $\Delta h_{0.9}[+1.50\text{\AA}]$  are for 56 residue proteins compared to 57 residue proteins in all other cases (see Methods and materials) which results in  $T_m$ 's that are estimated to be about 2 °C higher than what would be expected for the corresponding 57 residue proteins based on the  $T_m$  difference between the 56 and 57 residue versions of G $\beta$ 1. The removal of the strained leucine at position seven (L7I) along with the increased hydrophobic burial generated by the Y3F and V39I mutations in  $\Delta h_{0.9}[0.00\text{\AA}]$  result in a protein that is measurable more stable than wild-type G $\beta$ 1. The extent of chemical shift dispersion in the 1D  $^1\text{H}$  NMR spectrum of each mutant was assessed to gauge each protein's degree of native-like character (Fig. 5). All of the mutants, except  $\Delta h_{0.9}[+1.50\text{\AA}]$ , have NMR spectra with chemical shift dispersion similar to that of G $\beta$ 1 suggesting that the proteins form well-ordered structures.  $\Delta h_{0.9}[+1.50\text{\AA}]$